SOA⟨X⟩ User's Guide

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Contents

1 Introduction

This manual describes SOAX program and teaches how to use it to segment biological network structures from 3D image data. It also illustrates how to get quantitative measurements based on the segmentation results. Figure 1 shows the user interface of SOAX program. For more information regarding the method, please refer to [1, 2]. Here we use snakes and SOACs interchangeably for the centerline curves.
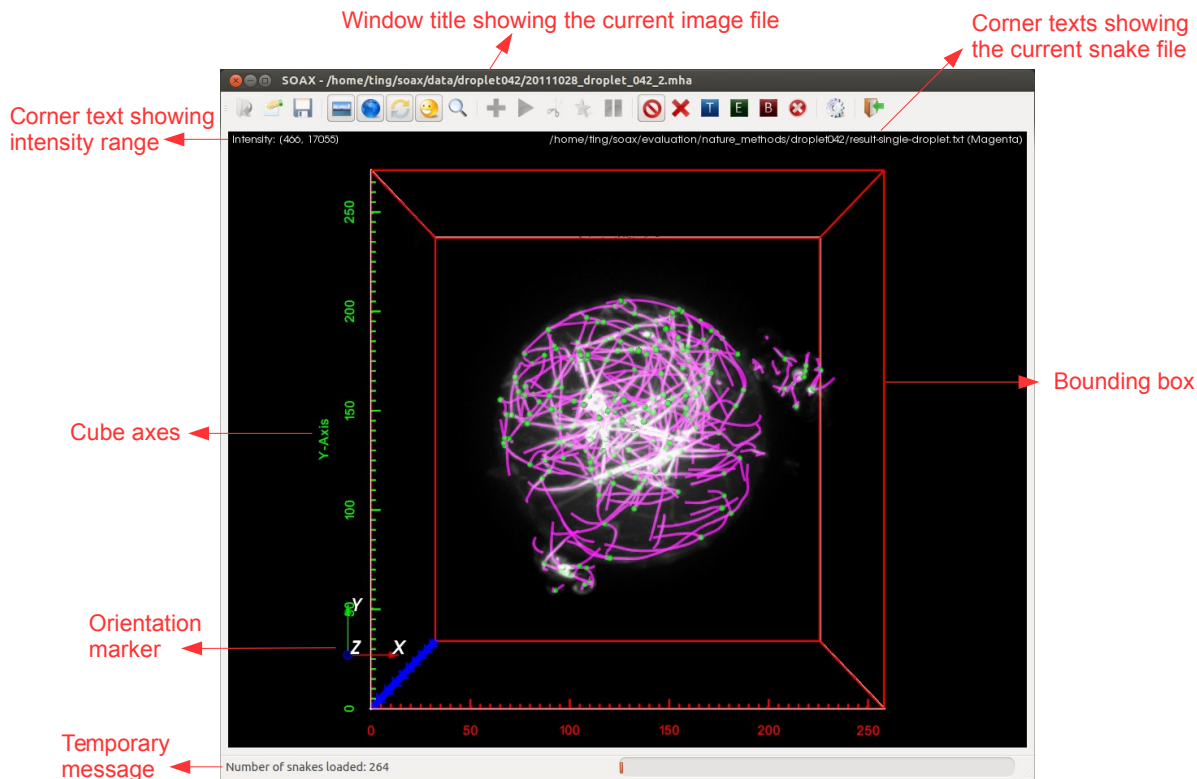
Figure 1: SOAX User Interface

2 Segmentation Workflow

Segmenting a network structure using SOAX GUI usually needs the following steps:

1. Open an image (| File > Open Image) in TIFF (*.tif, *.tiff) or MetaImage (*.mhd, *.mha) format. The program remembers the last opened directory.

2. (Optional) Save an isotropic image (File > Save as Isotropic Image) if the original image has different voxel spacing in z-axis than that of x and y-axis. This prompts the ratio between z and xy spacing as in Figure 2. Then the interpolated image can be saved in TIFF or MetaImage format. After the isotropic image is saved, close the current session (| File > Close Session) and load the new isotropic image instead.

3. Set snake parameters in the Parameter Settings panel (| Tools > Parameters). One can also directly load a text parameter file (| File > Load Parameters). The loaded values can be changed in the panel afterwards (see Figure 3).
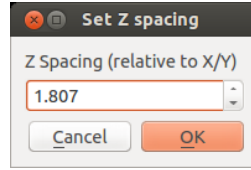
Figure 2: Specify z spacing relative to xy spacing.
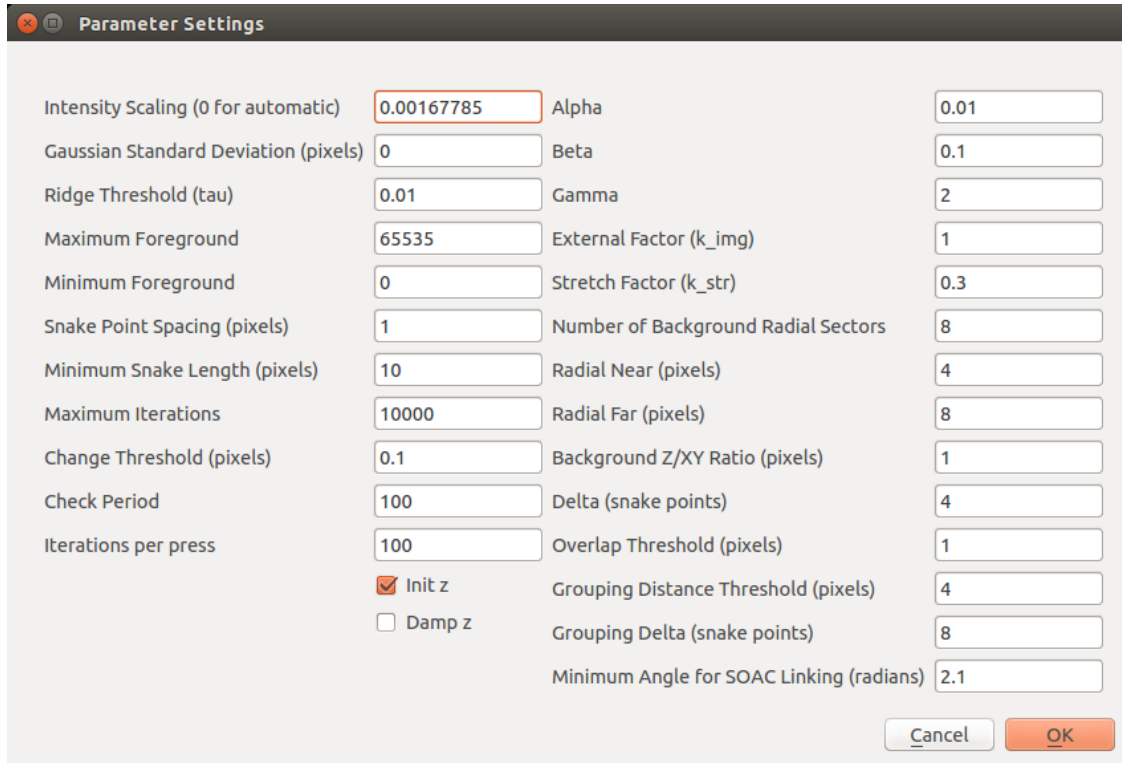
Figure 3: Parameter Settings panel.

4. Initialize snakes (➕ | Process > Initialize Snakes). One can change the parameters and then re-initialize snakes without closing the current session.

5. Evolve snakes (▶ | Process > Deform Snakes).

6. Cut snakes (✂ | Process > Cut Snakes at Junctions)

7. Group snakes (⭐ | Process > Group Snakes)

8. (Optional) Save snakes (💾 | File > Save Snakes) in a text file or save in JFilament [3] compatible format (File > Save JFilament Snakes).

9. (Optional) Close current session (🚪 | File > Close Session) and go back to step 1. The current parameter setting persists across sessions.

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3   Snake File Structure

The example snake file is shown below. Junction coordinates are appended at the end of a snake file. The local background image intensities are computed by averaging the pixel intensities sampled in the local annulus neighborhood (see "Number of Background Radial Sectors, Radial Near R_{near} and Radial Far R_{far}" in Section 4.3). Moreover, only pixel intensities higher than "Minimum Foreground" are considered.

Figure 4: Snake file structure.

4   SOAX Parameters

Parameters can be saved in a text file (File > Save Parameters) and loaded by (📝 | File > Load Parameters). A parameter file specifies all the parameters listed below and its content is the same as the parameter settings

part of a snake file (see Figure 4).

Two most important parameters are "Ridge Threshold τ" and "Stretch Factor k_{str}". The former controls how many snake are initialized and the latter controls how much snakes elongate.

4.1   SOAC Initialization

Intensity Scaling multiplies the intensity of every pixel such that the range of rescaled intensities lie roughly between 0.0 and 1.0. This allows using a standard range of α, β and other parameters below. Leave fixed for a given set of images. Set this value to 0 for automatic scaling, where the maximum intensity is scaled to exact 1.0.

Gaussian std σ (in pixels) controls the amount of Gaussian smoothing in the computation of image gradient (See Section 2.1 and Equation 3 in [2]). Set $\sigma < 0.01$ to disable smoothing.

Ridge Threshold τ (also "grad-diff") controls the number of initialized snakes (see Section 2.2.1 in [2]). Decrease this value to initialize more snakes.

Minimum and Maximum Foreground specifies the range of intensities intended for extraction. Snakes are not initialized where the image intensity is below "background" or above "foreground". During evolution, stretching force is zero when the intensity at a snake tip is outside this range.

Snake Point Spacing δ (in pixels) specifies the spacing between consecutive snake points (see the end of Section 2.1 in [2]).

Init z toggles the initialization of snakes along z-axis. Uncheck it to eliminate snakes that are perpendicular to filaments due to anisotropic PSF with larger spreading along z-axis.

4.2   SOAC Convergence

Minimum Snake Length (also "minimum-size", in pixels) specifies the minimum length of a resultant snake. Increase the value to eliminate hair-like snake structures as well as to avoid the snakes picking up actin patches in yeast images.

Maximum Iterations specifies the maximum number of iterations allowed in each snake evolution.

Check Period specifies the cycle of checking for convergence in number of iterations. A value of 100 means a snake is checked for convergence every 100 iterations. (see the last sentence of Section 2.1 in [2]).

Change Threshold (in pixels) specifies the threshold of change for a snake to be converged. A value of 0.05 means a snake is converged if every snake point drifts less than 0.05 pixels since last check for convergence (see the last sentence of Section 2.1 in [2]).

4.3   SOAC Evolution

Alpha α is the weight of first order continuity of snake (see Equation 1 in [2]). This term describes the energy penalty to elongate snakes. For images with dim linear structures and bright spots, one may want to use small value of alpha. Default is 0.01.

Beta β is the weight of second order continuity of snake (see Eq.1 in [2]). This term describes the snake bending energy penalty. Use larger value to make snakes more straight. Default is 0.1.

Gamma γ controls the step size of the iterative process of snake evolution. The smaller gamma is, the faster snakes converge but the result is less accurate (see Equation 6 in [2]).

External Factor k_{img} (also "weight") is the weight of image forces (image gradient) (see Equation 2 in [2]). Increasing this value to make snakes follow more closely the local shape of filaments.

Stretch Factor k_{str} (also "stretch") is the weight of stretching force (see Equation 2 in [2]). Increasing this value to stretch snakes more in case of under-segmentation.

Number of Background Radial Sectors, Radial Near R_{near}**, Radial Far** R_{far} define the local annulus from which magnitude of stretching forces (see Section 2.1.1 and Figure 3 in [2]) and local image SNR are computed.

Background Z/XY Ratio defines the anisotropy of the PSF of microscope. It is the spreading of PSF along z-axis relative to that of x and y-axis. Set this parameter to fix the anisotropy in the background intensity calculation. Default is 1.0.

Delta specifies the number of snake points for determining the tangential direction at the tip. Default is 4.

Overlap Threshold D_{min} is the distance threshold that snakes are considered overlapping.

Grouping Distance Threshold specifies the maximum distance that two T-junctions formed after snake evolution can be clustered into one clustered junction for grouping. Large values may help collapsing unwanted T-junctions.

Grouping Delta specifies the number of snake points for determining the tangent direction of snake branches of a clustered junction (see Equation 9 in [2]). Default is 8.

Minimum Angle for SOAC Linking θ (in radians) is the angular threshold for grouping snakes. The angle between the tangent directions of two snake branches in a clustered junction must be greater than this value to be grouped. Default is $2\pi/3$.

Damp z toggles the suppression of snake evolution along the z-axis. This may be useful when anisotropy in PSF along z becomes a problem.

Iteration per press specifies the number of iterations for evolving a single snake (▐▐ | Process > Deform One Snake).

5   Image and Snake Viewing

5.1   Mouse Control

Gestures of mouse navigation in the rendering window are:

Button	On Slice Planes	Anywhere else
Left	Show image voxel location and intensity	Change viewing angle
Middle	Change window (horizontal drag) or level (vertical drag) on the selected slice plane	Translate image at a given viewing angle
Right	Move the selected slice plane along its normal direction	Zoom in/out at a given viewing angle

5.2   Image Viewing

Image can be inspected by three orthogonal slice planes (🖼 | View > Slice Planes) and Maximum Intensity Projection (MIP) rendering (🔵 | View > MIP Rendering). Additionally, there are *Orientation Marker* (View > Orientation Marker) showing current image orientation, *Corner Texts* (View > Corner Texts) displaying image intensity range and loaded snake file names, *Bounding Box* (View > Bounding Box) delimiting the image volume, and *Cube Axes* (View > Cube Axes) acting as an image ruler (see Figure 1). All these can be toggled on/off individually.

5.3 Snakes Viewing

There are four exclusive modes for snake viewing. Only one mode is enabled at a time. Junctions shown in green spheres (😊 | View > Junctions) can also be toggled on/off.

🔄 | View > Snakes	Default mode for snake display.
🔍 | View > Show Snakes Locally	Display snake locally within a small range of selected slice plane. The clipped snakes are updated accordingly as the slice plane moves.
View > Color Snakes by Azimuthal Angle	Color snakes based on its orientation (azimuthal angle in spherical coordinate system)
View > Color Snakes by Polar Angle	Color snakes based on its orientation (polar angle in spherical coordinate system)

5.4 Viewing Options
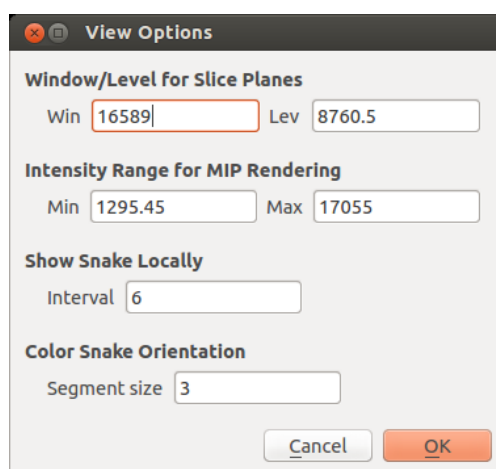
Options can be accessed via View > Options (Figure 5).

Figure 5: Options related to image and snake viewing.

View Options		
Slice Planes	Window	Adjusts image contrast. The default is $I_{max} - I_{min}$, where I_{max} and I_{min} are the maximum and minimum intensities in the image, respectively.
	Level	Adjusts image brightness. The default is $(I_{max} + I_{min})/2$.
MIP Rendering	Min	Minimum image intensity shown in MIP rendering. Intensities less than *Min* are displayed completely transparent. The default is $I_{min} + 0.05 * (I_{max} - I_{min})$. The opacity increases linearly for intensities between *Min* and *Max*.
	Max	Image intensity saturated in MIP rendering. Intensities greater than *Max* are displayed completely opaque and white. The default is I_{max}.
Show Snakes Locally	Interval	Only snakes that lie between position of selected slice plane $\pm$ *Interval* are displayed (see Figure 6(a)). This helps reduce clutter when viewing dense snakes.
Color Snake Orientation	Segment size	Minimum size of a uniform-color snake segment.

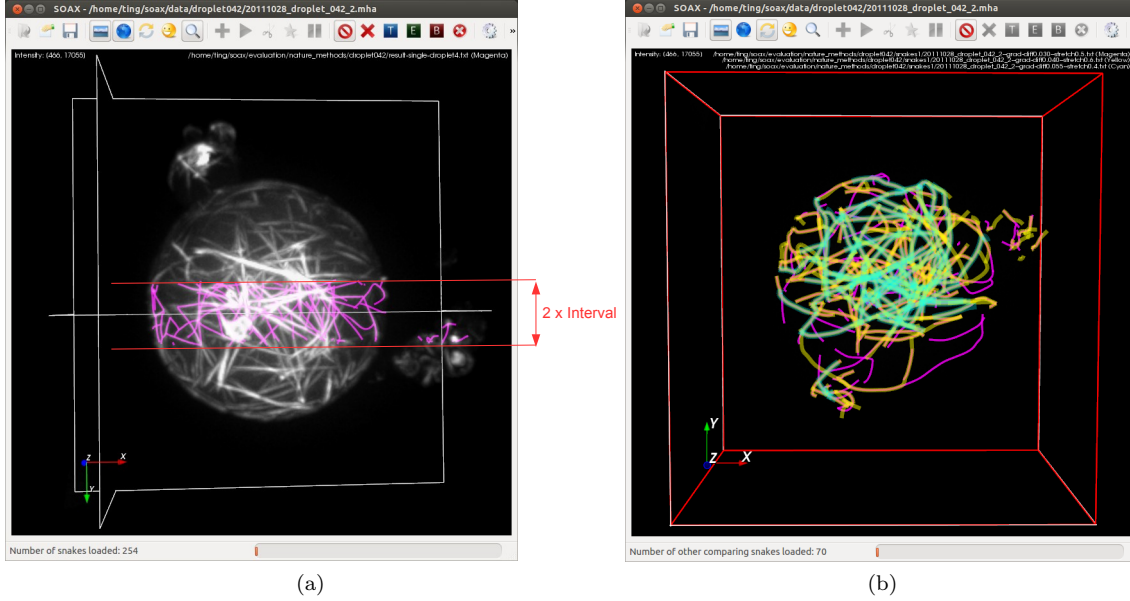(a)   (b)

Figure 6: (a) Showing snake locally around a certain slice plane. (b) Visually comparing three different set of snakes.

5.5 Comparing Snakes

Up to three set of snakes can be loaded simultaneously for comparison (see Figure 6(b)). Start by loading the first result (File > Load Snakes), then the second (File > Compare Snakes) and the third (File > Compare Another Snakes). The loaded snake file names are displayed on the upper right corner of the rendering window, which can be turned on/off (View > Corner Texts). If any other result needs to be compared, one can directly load it using any one of these three actions without closing the current session (| File > Close Session).

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6 Snake Editing

No Editing (⃠ | Edit > Edit Mode > Normal Mode). Individual snake inspection.

1. Toggle slice planes off (▨ | View > Slice Planes)
2. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)
3. Select individual snake by left mouse click
4. Check the console window accompanying SOAX program for snake information
5. (Optional) Deselect snake by right mouse click

Note: The output in the console window includes snake ID, whether the snake is open curve or not, length, spacing, and point position with corresponding intensity, where the first column is index, second to fourth are x, y, z coordinates, fifth column shows snake point intensities.

Deleting Snakes Delete one or more snakes from result.

1. Turn Delete Snake Mode on (✖ | Edit > Edit Mode > Delete Snake Mode)
2. Toggle slice planes off (▨ | View > Slice Planes)
3. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)
4. Left click to select one or more snakes (snakes can be selected simultaneously); right click to deselect
5. Execute (SPACE | Edit > Edit Snake)

Trimming Snake Tip Cut part of a snake from its tip.

1. Turn Trim Tip Mode on (T | Edit > Edit Mode > Trim Tip Mode)
2. Toggle slice planes off (▨ | View > Slice Planes)
3. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)
4. Left click a point on a snake to indicate from where the snake will be cut off. A red sphere will mark the point. Update the point by another left click. Right click to deselect the current point.
5. Execute (SPACE | Edit > Edit Snake)

Extending Snake Tip Extending a snake tip to a new location.

1. Turn Extend Tip Mode on (T | Edit > Edit Mode > Extend Tip Mode)
2. Toggle slice planes off (▨ | View > Slice Planes)
3. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)
4. Left click a point on a snake to indicate from which end to extend. A red sphere will mark the point. Update the point by another left click. Right click to deselect the current point.
5. Toggle slice planes back on (▨ | View > Slice Planes)
6. Left click on any one of the slice planes to indicate the desired position to extend to. The slice planes can be moved by holding and dragging the right mouse button. Update the point by another left click.
7. Execute (SPACE | Edit > Edit Snake)

8. (Optional) To remove the introduced kink, evolve the edited snake by repeatedly clicking ▮▮. One click evolve *Iterations per press* iterations. The value can be changed through the parameter setting panel (⚙ | Tools > Parameters). Other parameter controlling snake evolution can also be adjusted.

Modifying Snake Body Modify part of a snake to let it go through a new point.

1. Turn Trim Body Mode on (🅣 | Edit > Edit Mode > Extend Tip Mode)

2. Toggle slice planes off (🖼 | View > Slice Planes)

3. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)

4. Consecutively left click two points on a snake to indicate the part that needs to be modified. Two red spheres will mark the interval and the snake will turn cyan. Update points by other left clicks (The program updates the first and second point alternately). Right click to deselect a point.

5. Toggle slice planes back on (🖼 | View > Slice Planes)

6. Left click on any one of the slice planes to indicate the desired position to modify the interval indicated by the first two points. The slice planes can be moved by holding and dragging the right mouse button. Update the point by another left click.

7. Execute (SPACE | Edit > Edit Snake)

8. (Optional) To remove the introduced kink, evolve the edited snake by repeatedly clicking ▮▮. One click evolve *Iterations per press* iterations. The value can be changed through the parameter setting panel (⚙ | Tools > Parameters). Other parameter controlling snake evolution can also be adjusted.

Delete Junctions Delete green junction points from results.

1. Turn Delete Junction Mode on (❌ | Edit > Edit Mode > Delete Junction Mode)

2. Toggle slice planes off (🖼 | View > Slice Planes)

3. (Optional) Toggle MIP rendering off (🌐 | View > MIP Rendering)

4. Left click to select one or more junctions (junctions can be selected simultaneously); right click to deselect

5. Execute (SPACE | Edit > Edit Snake)

User can always save the snakes and junctions after editing (💾 | File > Save Snakes).

7 Quantitative Analysis

User can perform quantitative analysis using the resultant snakes in the Analysis menu [1]. Snakes are either loaded from file (File > Load Snakes) or at least finish the evolution process (Step 5 of the Segmentation workflow). Currently SOAX supports the computation of spherical orientation, radial orientation, filament density, curvature, and length. User can specify parameters in the Analysis > Options (Figure 7). All the output are CSV formatted files.

Spherical Orientation Analysis > Compute Spherical Orientation generates a file containing the polar and azimuthal angles of filament local tangents inside a spherical confinement. A user needs to specify the x, y, z coordinates of the Center, the Radius of spherical confinement, and a Inside Ratio. Only local tangents that are within distance of Inside Ratio * Radius to the Center are considered. If the Exclude points near image boundary is checked, snake points that are within 2 pixels from the image boundary are excluded from the computation.
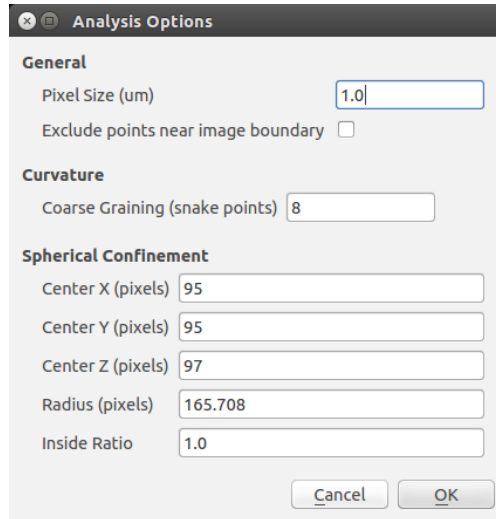
Figure 7: Specifying analysis parameters in the Analysis Options panel.

Radial Orientation Analysis > Compute Radial Orientation generates a file containing angles between filament local tangents and the outward radial direction with respect to the radius in a spherical confinement. User needs to specify the x, y, z coordinates of the Center and Pixel Size.

Filament Density and Intensity Analysis > Compute Point Density generates a file containing the snake point density and intensity as well as the voxel intensity with respect to the radius in a sperical confinement. User needs to specify the x, y, z coordinates of the Center, the Radius of spherical confinement, the Inside Ratio and the Pixel Size. Only points and voxels that lie within Inside Ratio * Radius are considered.

Filament Curvature Analysis > Compute Curvature generates a file containing curvature (μm^{-1}) of filaments. User can specify the Pixel Size and Coarse Graining. If the Exclude points near image boundary is checked, snake points that are within 2 pixels from the image boundary are excluded from the computation.

Filament Length Analysis > Compute Snake Length generates a file containing lengths (μm) of all the snakes. User can specify the Pixel Size.

All in One Analysis > Compute All generates all the above analysis files in the specified folder.

8 Miscellaneous

8.1 Viewing Sequential Evolution

The sequential snake evolution process can be displayed for an inquisitive mind. Instead of ▶ | Process > Deform Snakes, one can use ⇄ | Process > Deform Snakes in Action after snake initialization (Step 4 in the Segmentation Workflow).

8.2 Managing Viewing Angle

Current viewing angle can be loaded (Tools > Load Viewpoint) from a text file (*.cam) and saved (Tools > Save Viewpoint) as a text file (*.cam).

8.3 Program Snapshot

One can take snapshot (Tools > Take Snapshot) of the current rendering window and save as an image file (*.png). The program also automatically writes a copy in TIFF format. *Note: one needs to specify the ".png" suffix when prompted the file name.*

8.4 Viewing Local Image SNR

One can also view the local image SNR by clicking on the snakes. The console window outputs information on the selected snake which includes the local image SNR value. To change the size/position of local background annulus, one can go to ⚙ | Tools > Parameters and change the values of "Radial Near" and "Radial Far".

8.5 Extraction on 2D Images

The procedure of extraction on 2D images are the same as in 3D.

9 Commandline Usage

9.1 Running SOAX in batch mode

COMMAND
batch_soax – run SOAX in batch mode; sweep the parameter space of τ and k_{str}

SYNOPSIS
./batch_soax -i FILE/DIR -p FILE -s FILE/DIR [--ridge START STEP END] [--stretch START STEP END] [--invert]

DESCRIPTION
-i, --image FILE/DIR   Directory or path of input isotropic images (use File > Save as Isotropic Image to resample the image to be isotropic)

-p, --parameter FILE   Path of default parameter file

-s, --snake FILE/DIR   Directory or path of output snake files

--ridge START STEP END   (Optional) Range of ridge threshold τ

--stretch START STEP END   (Optional) Range of stretching factor k_{str}

--invert   (Optional) Invert image intensity

-h, --help   (Optional) Display help information

-v, --version   (Optional) Display program version

EXPLANATION
Turn on the --invert option if the foreground filaments are black while the background is white.

EXAMPLE

Extracting a set of images using the same parameters
./batch_soax -i ../input-image-dir/ -p ../parameters/default.txt -s ../result-snakes/

Sweeping parameter space on a single image
./batch_soax -i ../data/image.mha -p ../parameters/parameter.txt -s ../result-snakes/ --ridge 0.001 0.005 0.1 --stretch 0.1 0.1 1.0

Sweeping parameter space on multiple images
./batch_soax -i ../input-image-dir/ -p ../parameters/parameter.txt -s ../result-snakes/ --ridge 0.001 0.005 0.1 --stretch 0.1 0.1 1.0

9.2 Generation of Candidate Optimal Extractions

COMMAND

`best_snake` – generate filenames of candidate optimal extractions

SYNOPSIS

`./best_snake -i FILE -s DIR -o FILE [-n ARG] [-f ARG] [-t START STEP END] [-c START STEP END] [-e FILE] [-g FILE]`

DESCRIPTION

`-i, --image, FILE`   Path of input isotropic image (use File > Save as Isotropic Image to resample the image to be isotropic)

`-s, --snake DIR`   Directory of resultant SOAC files

`-o, --output FILE`   Path of output file that contains filenames of candidate extractions

`-n, --rnear ARG`   (Optional) Inner radius R_{near} of local background annulus (default: 4)

`-f, --rfar ARG`   (Optional) Outer radius R_{far} of local background annulus (default: 8)

`-t, --t-range START STEP END`   (Optional) Range of low SNR threshold (default: 1.0 0.1 3.0)

`-c, --c-range START STEP END`   (Optional) Range of penalizing factor (default: 1.0 0.1 3.0)

`-e, --error FILE`   (Optional) Path of the output "tc-candidate-error" file

`-g, --ground-truth FILE`   (Optional) Path of the ground truth snake file

`-h, --help`   (Optional) Display help information

`-v, --version`   (Optional) Display program version

EXPLANATION

The "tc-candidate-error" file lists the optimal extraction at each pair of (t, c) found by the F-function. If ground truth (-g option) is provided, its corresponding error measurements (vertex error and Hausdorff distance) are also written.

Example

`./best_snake -i input-image-dir/image.mha -s extraction-results/ -o candidate-results.txt -n 4 -f 8 -t 1.0 0.1 10.01 -c 1.0 0.1 10.01 -g ground-truth-snake-dir/gt-snake.txt -e tc-candidate-error.txt`

9.3 Batch Computing SOAC Lengths

COMMAND

`batch_length` – Compute SOAC lengths (in pixels) from multiple SOAC files.

SYNOPSIS

`./batch_length -i DIR [-o FILE]`

DESCRIPTION

`-i, --image, DIR`   Directory of SOAC files.

`-o, --output FILE`   (Optional) Output file path (default: "batch_length_output.csv").

`-h, --help`   (Optional) Display help information

`-v, --version`   (Optional) Display program version

EXAMPLE

`./batch_length -i resultant-soacs/ -o analysis-results/lengths.csv`

9.4 Batch Resampling TIFF Images

COMMAND

    `batch_resample` – Resample TIFF images to have isotropic voxel size.

SYNOPSIS

    `./batch_resample` <u>`INPUT DIR`</u> <u>`OUTPUT DIR`</u> <u>`Z SPACING`</u>

Example

    `./batch_resample input-images/ iso-images/ 2.88`

10 Release Notes

- Version 3.5.9

  1. Added Gaussian smoothing for 2D images;

- Version 3.5.8

  1. Added an analysis option to exclude points near image boundary;
  2. Updated column width of snake file;
  3. Several bug fixes;

- Version 3.5.7

  1. Added Analysis > Compute All to generate all analysis files in the specified folder;
  2. Added Inside Ratio (default = 1.0) in Analysis dialog; changed the default radius to be half of image diagonal so that all snakes points are included for analysis by default;
  3. Fixed the problem that Intensity Scaling is truncated by ParametersDialog; now it shows 0 in Parameters dialog if it is set to 0;
  4. Added batch resample commandline utility.
  5. Added local background output in the snake file.

- Version 3.5.6

  1. Texture interpolation on slicing planes is turned off by default;
  2. Added batch length computation for SOACs;
  3. Fixed disappearing of snakes, volume rendering, and slicing planes when "Compare Snakes" after "Load Snakes";

- Version 3.5.5

  1. First public version;

- Version 3.5.4

  1. Improved default parameters;
  2. Added progress bar when SOACs are deformed in action;

- Version 3.5.3

  1. Added support for 2D images;
  2. Capability to generate a set of candidate optimal extractions from a pool of extraction results;

3. Output average local image SNR of a user selected SOAC;

- Version 3.5.2

    1. Fixed memory leak when loading snakes;
    2. Fixed two bugs (reset of linear solvers and external force) of "close current session";
    3. Fixed the bug of applying stretch factor twice (should be once);
    4. Fixed the bug of applying snake tip intensity 8 times (should be once);
    5. Other minor tweaks such as making the format of snake file denser.

- Version 3.5.1

    1. Fixed a buffer overflow bug during SOAC initialization;
    2. Automatic determining the appearance of junction size depending on image size;
    3. Less memory leaks;

References

[1] Ting Xu, Dimitrios Vavylonis, Feng-Ching Tsai, Gijsje Koenderink, Wei Nie, Eddy Yusuf, I-Ju Lee, Jian-Qiu Wu, and Xiaolei Huang. "SOAX: a software for quantification of 3D biopolymer networks". In: *Scientific Reports* 5.9081 (2015).

[2] Ting Xu, Dimitrios Vavylonis, and Xiaolei Huang. "3D actin network centerline extraction with multiple active contours". In: *Medical Image Analysis* 18.2 (2014), pp. 272–284. ISSN: 1361-8415.

[3] Matthew B. Smith, Hongsheng Li, Tian Shen, Xiaolei Huang, Eddy Yusuf, and Dimitrios Vavylonis. "Segmentation and tracking of cytoskeletal filaments using open active contours". In: *Cytoskeleton* 67.11 (2010), pp. 693–705. ISSN: 1949-3592.